UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

November 14, 2006

**MEMORANDUM**

**Subject:** Efficacy Review for VigorOx® Liquid Sanitizer and Disinfectant, EPA Reg. No. 65402-1; DP Barcode: D332235.

**From:** Ibrahim Laniyan, Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

**Thru:** Tajah Blackburn, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510P)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

**To:** Karen Leavy / Marshall Swindell
Regulatory Management Branch I
Antimicrobials Division (7510P)

**Applicant:** FMC Corporation
Peroxygens Division
1735 Market Street
Philadelphia, PA 19103

**Formulation from the Label:**

| Active Ingredient | % by wt. |
|---|---|
| Peroxyacetic Acid | 5.1 % |
| Hydrogen Peroxide | 21.7 % |
| Inert Ingredients: | 73.2 % |
| Total | 100.0 % |

## I. BACKGROUND

The product, VigorOx® Liquid Sanitizer and Disinfectant (EPA Reg. No. 65402-1), is an EPA-approved disinfectant (bactericide), sanitizing rinse, sanitizer, and deodorizer for use on hard, non-porous surfaces in institutional, industrial, commercial, food processing, animal care, and hospital or medical environments. The applicant requested to amend the registration of this product to add sterilant claims. The applicant also requested to amend the registration of this product to add claims for effectiveness as a disinfectant against additional microorganisms, including Influenza A virus (H1N1 and H3N2 strains), Influenza B virus, Parainfluenza virus type 3, Avian infectious bronchitis virus, Avian reovirus, Infectious bovine rhinotracheitis virus, Infectious bursal disease virus, and Newcastle disease virus. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained EPA Form 8570-35 (Data Matrix), ten studies (MRID Nos. 469073-01 through 469073-10), Statements of No Data Confidentiality Claims for all ten studies, and the proposed label.

## II. USE DIRECTIONS

The product is designed for use in sterilizing porous and non-porous surfaces, such as laboratory equipment, manufacturing and packaging equipment, and veterinary equipment. Directions on the proposed label provided the following information regarding preparation and use of the product as a sterilant: Remove gross filth with a suitable detergent. Rinse with clean water. Prepare a use solution by adding 7.5 ounces of the product to 1 gallon of water (a 5.8% solution). Apply the use solution by spraying, sponging, or flooding. Allow surfaces to remain wet for 6 hours. Rinse food contact surfaces with potable or sterile water, followed by a sanitizing solution of the product (i.e., a 0.16% solution).

The product also is designed for disinfecting hard, non-porous surfaces such as bathroom fixtures, bed frames, cages, carts, chairs, coolers, counter tops, feeding and watering equipment, floors, garbage cans, kennel runs, racks, refrigerators, shelves, sinks, tables, and walls. The label indicates that the product may be used on hard, non-porous surfaces including: asphalt, glass, linoleum, plastic (e.g., polypropylene, polyethylene), porcelain, stainless steel, tile, and vinyl. Directions on the proposed label provided the following information regarding preparation and use of the product as a virucide: Remove gross filth from surfaces by cleaning with a detergent or suitable cleaning product. Rinse with clean water. Prepare a use solution by adding 2 2/3 ounces of the product to 5 gallons of water (a 0.42% solution). Apply the use solution by wiping, mopping, or as a coarse spray. Allow surfaces to remain wet for 5 minutes. Air dry.

## III. AGENCY STANDARDS FOR PROPOSED CLAIMS

**Sterilizers:** The AOAC Sporicidal Test is required for substantiating sterilizing claims. The following information applies to all products represented as sporicidal or sterilizing agents. Sixty carriers, representing each of 2 types of surfaces (porcelain penicylinders and silk suture loops), must be tested against spores of both *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) on 3 product samples representing 3 different product lots, one of which is at least 60 days old (240 carriers per sample; a total of 720 carriers). Any sterilizing

agent (liquid, vapor, or gas) that is recommended for use in a specific device must be tested by the AOAC Sporicidal Test in that specific device and according to the directions for use. Killing on all of the 720 carriers is required; no failures are permitted. Data to support sterilizing claims must be confirmed by tests conducted by a second, independent laboratory of the applicant's choice (other than the laboratory that developed the original data). The following minimal confirmatory data must be developed on one sample of the product: Thirty carriers with each of the 2 types of surfaces (silk suture loops and porcelain penicylinders) against spores of both *Bacillus subtilis* and *Clostridium sporogenes* (a total of 120 carriers) by the AOAC Sporicidal Test. These Agency standards are presented in DIS/TSS-9.

**Virucides:** The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least $10^4$ from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

**Supplemental Claims:** An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. These Agency standards are presented in DIS/TSS-2. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level. These Agency standards are also presented in DIS/TSS-2.

## IV. BRIEF DESCRIPTION OF THE DATA

### 1. MRID 469073-01 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus (H3N2)" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 24, 2006. Project Number A03529.

This study was conducted against Influenza A virus (H3N2) (Strain Hong Kong; ATCC VR-544) using cultures of Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories Inc.) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.FLUA.2 (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 493 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic

soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 51% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.75 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥5.25 $\log_{10}$** for both batches.

**2. MRID 469073-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Infectious Bursal Disease" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – February 24, 2006. Project Number A03528.**

This study was conducted against Infectious bursal disease virus (strain not specified; obtained from Solvay Animal Health) using cultures of Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.IBD (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 502 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 19.0°C at 54% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 19.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **4.5 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.0 $\log_{10}$** for both batches

Note: The applicant provided data for a failed trial set up on January 6, 2006. In that trial, the dried carrier control count ($TCID_{50}$/0.1 ml of $10^{3.0}$) was below the required number (at least $10^4$).Thus, the test was invalid. These data were not used to evaluate efficacy of the product. See Attachment I of the laboratory report.

**3. MRID 469073-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Infectious Bovine Rhinotracheitis" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – March 23, 2006. Project Number A03724.**

This study was conducted against Infectious bovine rhinotracheitis virus (Strain LA; ATCC VR-188) using cultures of rabbit kidney cells (obtained from ViroMed Laboratories Inc.) as the host system. Two lots (Lot Nos. 60124 35 and 60131 73) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.IBR (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 493 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 35% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. Rabbit kidney cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **4.5 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.0 $\log_{10}$** for both batches

**4. MRID 469073-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus (H1N1)" for VigorOx® LS & D, by Mary Miller. Study conducted at ATS Labs. Study completion date – November 2, 2005. Project Number A03311.**

This study was conducted against Influenza A virus (H1N1) (Strain A/New Caldonia/20/99; obtained from the Centers for Disease Control) using cultures of Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc.) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx® LS & D, were tested according to ATS Labs Protocol No. FMC04090105.FLUA (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 498 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 38% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored

periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.0 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.5 $\log_{10}$** for both batches

**5. MRID 469073-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Parainfluenza virus type 3" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – June 7, 2006. Project Number A03722.**

This study was conducted against Parainfluenza virus type 3 (Strain C243; ATCC VR-93) using cultures of MDBK cells (ATCC CCL-22; propagated in-house) as the host system. Two lots (Lot Nos. 60124 35 and 60131 73) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.PFLU (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 491 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 49% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. MDBK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 5 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.25 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.75 $\log_{10}$** for both batches

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided data for a failed trial set up on March 9, 2006. In that trial, the dried carrier control count (infectivity undetectable) was below the required number (at least $10^4$). Thus, the test was invalid. These data were not used to evaluate efficacy of the product. See Attachment I of the laboratory report.

**6. MRID 469073-06 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Newcastle disease virus" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – April 12, 2006. Project Number A03723.**

This study was conducted against Newcastle disease virus (Strain B1, Hitchner, or Blacksburg; ATCC VR-108) using cultures of chicken embryo fibroblast cells (obtained from

Charles River SPAFAS) as the host system. Two lots (Lot Nos. 60124 35 and 60131 73) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.NEW (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 500 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 18.6°C at 50% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 18.6°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 5% tryptose phosphate broth, 2.0 mM L-glutamine, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. Chicken embryo fibroblast cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.0 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.5 $\log_{10}$** for both batches.

Note: The applicant provided data for a failed trial set up on March 10, 2006. In that trial, the dried carrier control count ($TCID_{50}$/0.1 ml of $10^{3.25}$) was below the required number (at least $10^4$). Thus, the test was invalid. These data were not used to evaluate efficacy of the product. See Attachment I of the laboratory report.

**7. MRID 469073-07 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Infectious Bronchitis" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 23, 2006. Project Number A03530.**

This study was conducted against Avian infectious bronchitis virus (Strain Beaudette IB42; obtained from Solvay Animal Health) using embryonated chicken eggs (obtained from Charles River SPAFAS) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.AIBR (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 502 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 59% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in sterile phosphate buffer. Embryonated chicken eggs were inoculated intra-allantoically in quadruplicate with 0.1 ml of the dilutions. Eggs were candled daily to determine embryo viability, and any dead embryos were discarded. The eggs were incubated for 3 days at 34-38°C in 40-80% relative humidity. Embryos viable after incubation were considered negative for the test virus. Controls included those for dried virus count, toxicity, and neutralization. Viral and toxicity titers were

calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.25 $log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.75 $log_{10}$** for both batches

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

**8. MRID 469073-08 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza B virus" for VigorOx® Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 24, 2006. Project Number A03527.**

This study was conducted against Influenza B virus (Strain B/Hong Kong/5/72; ATCC VR-823) using cultures of Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc.) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.FLUB (copy not provided). A use solution was prepared by adding 1.0 ml of the product to 249.0 ml of 500 ppm AOAC synthetic hard water (titrated at 493 ppm; a 0.4% solution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 51% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.5 $log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥5.0 $log_{10}$** for both batches

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

**9. MRID 469073-09 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Reovirus" for VigorOx™ Liquid Sanitizer and Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 24, 2006. Project Number A03526.**

This study was conducted against Avian reovirus (Strain 2177; ATCC VR-2449) using cultures of chicken embryo fibroblast cells (obtained from Charles River SPAFAS) as the host system. Two lots (Lot Nos. 666952 and 679125) of the product, VigorOx™ Liquid Sanitizer and Disinfectant, were tested according to ATS Labs Protocol No. FMC04090105.AREO (copy not provided). A use solution was prepared by adding 1 ml of the product to 249 ml of 500 ppm AOAC synthetic hard water (titrated at 493 ppm; a 0.4% solution). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by

spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 54% relative humidity. For each lot of product, separate dried virus films were exposed to 2.0 ml of the use solution for 5 minutes at 20.0°C. After the contact period, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through individual Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, 10 µg/ml gentamicin, 100 units/ml penicillin, 5% tryptose phosphate broth, and 2.5 µg/ml amphotericin B. Chicken embryo fibroblast cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The average titer of the dried virus control was **5.0 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the average reduction in viral titer was **≥4.5 $\log_{10}$** for both batches

**10. MRID 469073-10 "Sporicidal Activity of Disinfectants," Test Organisms: *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584) for VigorOx® Liquid Sanitizer and Disinfectant, by Amy Jeske. Study conducted at ATS Labs. Study completion date – June 30, 2005. Project Number A02861.**

This study was conducted against *Bacillus subtilis* (ATCC 19659) and *Clostridium sporogenes* (ATCC 3584). Three lots (Lot Nos. 0000597969, 0000606469, and 000635264) of the product, VigorOx® Liquid Sanitizer and Disinfectant, were tested using the AOAC Sporicidal Activity of Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. One of the product lots (i.e., Lot No. 0000606469) was at least 60 days old at the time of testing. A use solution was prepared by adding 7.5 ounces of the product to 1 gallon of 400 ppm AOAC synthetic hard water (titrated at 402-406 ppm; a 5.85% solution). The stock culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Sixty (60) porcelain penicylinder carriers and sixty (60) silk suture loops were contaminated by immersion in a 72±4 hour old culture of the test organism, at a ratio of 1 carrier per 1 ml of broth. The carriers were vacuum-dried at ≥69 cm Hg for 24-96 hours. Five dried carriers were placed in tubes and exposed to 10.0 ml of the use solution for 6 hours at 20±1°C. After the contact period, each carrier was transferred to individual tubes containing 10 ml of Fluid Thioglycollate Medium with 0.1% sodium thiosulfate. After subculturing, the carriers were transferred to secondary subculture tubes containing Fluid Thioglycollate Medium with 0.07% Lecithin and 0.5% Tween 80. The subcultures were incubated for 21 days at 35-37°C, and then examined for growth. Tubes showing no growth were heat-shocked for 20 minutes at 80±2°C, re-incubated for 72±4 hours at 35-37°C, and again examined for growth. Controls included those for purity, sterility, viability, carrier population count, neutralization confirmation, and acid resistance at 2, 5, 10, and 20 minutes. The reported titers per inoculated carriers are: ***Bacillus subtilis* on Suture Loops $1.14 \times 10^5$, *Bacillus subtilis* on Penicylinders $1.09 \times 10^5$, *Clostridium sporogenes* on Suture Loops $1.17 \times 10^6$, *Clostridium sporogenes* on Penicylinders $2.00 \times 10^6$.**

Note: Testing was also conducted for a contact time of 0.25 hours at 60±1°C.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

## V. RESULTS

| MRID Number | Organism | Results | | | Dried Virus Control |
|---|---|---|---|---|---|
| | | | Lot No. 666952 | Lot No. 679125 | |
| 469073-01 | Influenza A virus (H3N2) | $10^{-1}$ to $10^{-8}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.75}$ $TCID_{50}$/0.1ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-02 | Infectious bursal disease virus | $10^{-1}$ to $10^{-6}$ dilutions | Complete inactivation | Complete inactivation | $10^{4.5}$ $TCID_{50}$/0.1ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-04 | Influenza A virus (H1N1) | $10^{-1}$ to $10^{-7}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.0}$ $TCID_{50}$/0.1ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-07 | Avian infectious bronchitis virus | $10^{-1}$ to $10^{-8}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.25}$ $LD_{50}$/0.1 ml |
| | | $LD_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-08 | Influenza B virus | $10^{-1}$ to $10^{-7}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.5}$ $TCID_{50}$/0.1ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-09 | Avian reovirus | $10^{-1}$ to $10^{-8}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.0}$ $TCID_{50}$/0.1ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| | | | **Lot No. 60124 35** | **Lot No. 60131 73** | |
| 469073-03 | Infectious bovine rhinotracheitis virus | $10^{-1}$ to $10^{-7}$ dilutions | Complete inactivation | Complete inactivation | $10^{4.5}$ $TCID_{50}$/0.1 ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-05 | Parainfluenza virus type 3 | $10^{-1}$ to $10^{-8}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.25}$ $TCID_{50}$/0.1 ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |
| 469073-06 | Newcastle disease virus | $10^{-1}$ to $10^{-7}$ dilutions | Complete inactivation | Complete inactivation | $10^{5.0}$ $TCID_{50}$/0.1 ml |
| | | $TCID_{50}$/0.1 ml | $\leq 10^{0.5}$ | $\leq 10^{0.5}$ | |

| MRID Number | Organism | Lot No. | Carrier Type | No. Exhibiting Growth/No. Tested |
|---|---|---|---|---|
| **Testing conducted for a contact time of 0.25 hours at 60±1°C** | | | | |
| 469073-10 | *Bacillus subtilis* | 0000597969 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000606469 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000635264 | sutures<br>penicylinders | 1° 0/60; 2° 1/60<br>1° 0/60; 2° 0/60 |
| | *Clostridium sporogenes* | 0000597969 | sutures<br>penicylinders | 1° 0/60; 2° 1/60<br>1° 0/60; 2° 0/60 |
| | | 0000606469 | sutures<br>penicylinders | 1° 0/60; 2° 2/60<br>1° 0/60; 2° 0/60 |

| | | | | |
|---|---|---|---|---|
| | | 0000635264 | sutures<br>penicylinders | 1° 0/60; 2° 1/60<br>1° 0/60; 2° 0/60 |
| **Testing conducted for a contact time of 6 hours at 20±1°C** | | | | |
| 469073-10 | *Bacillus subtilis* | 0000597969 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000606469 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000635264 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | *Clostridium sporogenes* | 0000597969 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000606469 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |
| | | 0000635264 | sutures<br>penicylinders | 1° 0/60; 2° 0/60<br>1° 0/60; 2° 0/60 |

## VI. CONCLUSIONS

1. The submitted efficacy data (MRID No. 469073-10) **do not currently support** the use of a 5.85% use solution of the product, VigorOx® Liquid Sanitizer and Disinfectant, as a sterilant against *Bacillus subtilis* and *Clostridium sporogenes* on both porous and non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load at 20±1°C for a contact time of 6 hours. **Results of confirmatory testing by a second, independent laboratory for one sample of the product were not provided**. Otherwise, efficacy data provided fully met DIS/TSS-9 standards. At least one of the product lots tested was at least 60 days old at the time of testing. Carrier population counts met the laboratory acceptance criterion of at least 1 x $10^4$ CFU/carrier. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. *Clostridium sporogenes* test spores (suture loops and penicylinders) showed resistance to acid for at least 2 minutes, in both primary and secondary subcultures. *Bacillus subtilis* test spores (penicylinders) showed resistance to acid for at least 2 minutes, in secondary subcultures only. *Bacillus subtilis* test spores (suture loops) showed resistance to acid for at least 2 minutes, in both primary and secondary subcultures.

Note: Test results **do not support** the use of a 5.85% use solution of the product as a sterilant for a contact time of **0.25 hours** at 60±1°C.

2. The submitted efficacy data **support** the use of a 0.4% use solution of the product, VigorOx® Liquid Sanitizer and Disinfectant, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 500 ppm hard water and a 5% organic soil load for a contact time of 5 minutes:

| | |
|---|---|
| Avian infectious bronchitis virus | MRID No. 469073-07 |
| Avian reovirus | MRID No. 469073-09 |
| Infectious bovine rhinotracheitis virus | MRID No. 469073-03 |
| Infectious bursal disease virus | MRID No. 469073-02 |
| Influenza A virus (H1N1) | MRID No. 469073-04 |
| Influenza A virus (H3N2) | MRID No. 469073-01 |

| | |
|---|---|
| Influenza B virus | MRID No. 469073-08 |
| Newcastle disease virus | MRID No. 469073-06 |
| Parainfluenza virus type 3 | MRID No. 469073-05 |

Recoverable virus titers of at least $10^4$ were achieved. Cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

## VII. RECOMMENDATIONS

1. The proposed label claims that a 5.8% use solution of the product, VigorOx® Liquid Sanitizer and Disinfectant, is effective as a sterilant in the presence of 400 ppm hard water and a 5% organic soil load with an exposure time of 6 hours. Data provided by the applicant **do not currently support** this claim. As discussed in the "Conclusions" section of this efficacy report, **the applicant must provide confirmatory data to support product effectiveness as a sterilant.**

2. The proposed label claims that a 0.42% use solution of the product, VigorOx® Liquid Sanitizer and Disinfectant, is an effective disinfectant against Avian infectious bronchitis virus, Avian reovirus, Infectious bovine rhinotracheitis virus, Infectious bursal disease virus, Influenza A virus (H1N1), Influenza A virus (H3N2), Influenza B virus, Newcastle disease virus, Parainfluenza virus type 3 on hard, non-porous surfaces in the presence of hard water and moderate organic soil for a contact time of 5 minutes. Data provided by the applicant **support** these claims.

3. The applicant must make the following changes to improve the proposed label:

- On page 2 under the "Directions for Use" section (left panel), change "**pasteurizes**" to read "**pasteurizers**."

- On page 3 under the "Surface Disinfection" section (right panel), change "**Avian infectious bronchitis**" to read "**Avian infectious bronchitis virus**," change "**Infectious bursal disease**" to read "**Infectious bursal disease virus**," and change "**Infectious bovine rhinitracheitis**" to read "**Infectious bovine rhinotracheitis virus**."

- On page 3 under the "Surface Disinfection" section (right panel), change "**tile**" to read "**glazed tile**" and change "**porcelain**" to read, "**glazed porcelain.**"

- On page 3 under the "Surface Disinfection" section (right panel), delete "**asphalt.**" **Asphalt is a porous surface.**

- On page 3 under the "Surface Disinfection" section (right panel), clarify information provided for virucidal disinfection by changing "**hard water**" to read "**500 ppm hard water**" and "**moderate organic soil**" to read "**moderate organic soil (tested as 5% serum).**"

- On page 4 (left panel) under the "Disinfection and Deodorizing of Animal Housing Facilities . . ." section (item 4), change "**rinse with water**" to read, "**rinse with potable water.**"